Signaling in plants during induction of resistance against pathogens

H. Shekar Shetty and Vasanthi U. Kumar*

In plants, initial pathogen infection induces resistance against further infection. The resistance thus induced can be systemic, long lasting and effective against a broad spectrum of pathogens. This response is termed as 'systemic acquired resistance' (SAR). It is an important component of plant defense against pathogen infection. It offers protection against a broad spectrum of micro-organisms similar to immunization in mammals. In recent years, tremendous progress has been made in understanding the molecular events that take place in the initiation and maintenance of SAR. Development of resistance is correlated with the accumulation of salicylic acid (SA) as well as the expression of a number of pathogenesis-related (PR) proteins. The role of systemic signals like salicylic acid, ethylene, jasmonates and electrical signals linking SAR and defense responses are detailed. The underlying mechanisms of immunization in plants and animals are discussed with special emphasis on the role of receptors and secondary messengers.

PLANTS, like animals, are constantly exposed to pathogen attack and different types of relationships, viz. non-interaction, beneficial interaction and harmful interaction, exist between plants and microbes. The final outcome of such interactions is generally controlled by the genetic composition of the host and also the environmental conditions influencing the pathogen attack[1]. Several lines of evidence have shown that all plants respond to a pathogen infection by the induction of an array of defense compounds[2], the time and degree of expression of which differentiates a susceptible plant from a resistant one[3]. The faster response to pathogen infection results in the enhanced resistance in an otherwise susceptible plant. Induction and enhancement of a plant's own defence mechanism without genetic manipulation, in response to an extrinsic stimulus is called 'induced resistance'. It is a biological plant protection in which the plant is the target for modification and not the pathogen. Here protection is based on the stimulation of defense mechanisms through metabolic changes that enable plants to defend themselves more effectively. By definition, it is the opposite of constitutive resistance mechanism and is also not induced by wounding or osmotic stress[4]. The induced resistance usually needs a time lag period for development of resistance, non-specific with respect to the inducing agent or resultant biological spectrum of activity and effective for several weeks to months[4]. Functionally, induced resistance can be differentiated into systemic acquired resistance (SAR)[5] and localized acquired resistance (LAR)[6]. SAR results from a low level of persistent metabolic stress caused by infection or chemical treatment rather than a specific response to the inducing agent and protects parts of the plant distinct from the treated area. LAR is detected only in areas immediately adjacent to the site of attempted penetration by the pathogen and is often accompanied by rapid collapse and dissociation of tissue (hypersensitivity) and with increase in phytoalexin (Figure 1). It appears to be due to a natural consequence of incompatible interaction between plants and pathogens and restricts to the site of prior inoculation[4]. The methods used to induce systemic resistance have been more complex than those used for localized resistance because SAR involves two separate inoculations with variations in space and time that are more difficult to quantify[6].

The natural phenomenon of resistance called 'plant immunity' had been recognized as early as 1901 (refs 7 and 8). The first review[9] on alterations of resistance after infection includes many descriptions of 'acquired physiological immunity' in plants. However, detailed analysis of induced resistance started only in 1960s after reproducible biological models were developed[5,6]. Since then, the efficiency of induced resistance in controlling plant diseases has been demonstrated in more than 25 crops that include bean, peas, apple, potato, cucumber, tomato, melons and cereals against a broad spectrum of pathogens like bacteria, fungi and viruses[10–15]. Induced resistance and the accompanying molecular events are much better characterized in dicots than in monocots[16]. In barley and wheat-induced resistance occurs only locally[16], while in rice[17] and pearl millet[10] it has been

The authors are in the Downy Mildew Research Laboratory, Department of Applied Botany and Biotechnology, University of Mysore, Mysore 570 006, India
*For correspondence. (e-mail: seedpath@bgl.vsnl.net.in)

reported to occur systemically. Induced resistance in monocots appears to be the result of complex processes in plant metabolism[16]. While increased activities of chitinases and glucanases are often correlated with systemic-induced resistance in dicots[18,19], these correlations are not shown clearly for monocots[16,20].

Acceleration of plant defense can be achieved by various biotic and abiotic agents[4,12,21–23]. In the last few years, application of resistance elicitors derived from various sources[24] has proved to protect crop plants in an ecofriendly manner. There are many reports that demonstrate the operation of induced resistance using either the pure form or crude mixture of biotic and/or abiotic elicitors[24,25]. Induction of SAR by seed treatment with inducers like chitosan[26] and benzothiadiazole[14] have been proved to be a practical approach to deliver the benefits of SAR and offer an advantage over the other control measures of easy application under commercial agricultural conditions.

SAR appears to be the result of several mechanisms which together are effective against a wide range of micro-organisms. It has been proposed that induced resistance is mediated by endogenous signal(s) that are produced in the infected leaf and translocated in the phloem to the other parts where it activates resistance mechanisms[1,27]. Signal transduction pathway (STP) that is initiated locally at the site of pathogen attack[21] leading to SAR, correlate with systemic accumulation of several defense responses. Induction of a set of classes of mRNAs that are coordinately induced with the onset of SAR has been characterized as a set of gene families called *SAR* genes[28]. SAR is associated with the expression of a number of genes, including pathogenesis-related genes, during the establishment of the resistant state[29–32]. Signaling mechanism leading to the observed differential temporal and spacial distribution of various components of the plant's defense response is complex and involves more than one pathways (Figure 2) and can be achieved through alterations in the morphology, biochemistry and developmental processes. Recent interest in the study of signal transduction has suggested the involvement of various signal molecules in the activation of a resistance state in plants. Knowledge of STP during a plant's response to pathogen attack is central to the understanding of disease susceptibility and resistance, and for applications in agricultural production and crop protection. This review summarizes the current status of knowledge in signaling that takes place in host plants during response to induction of resistance.

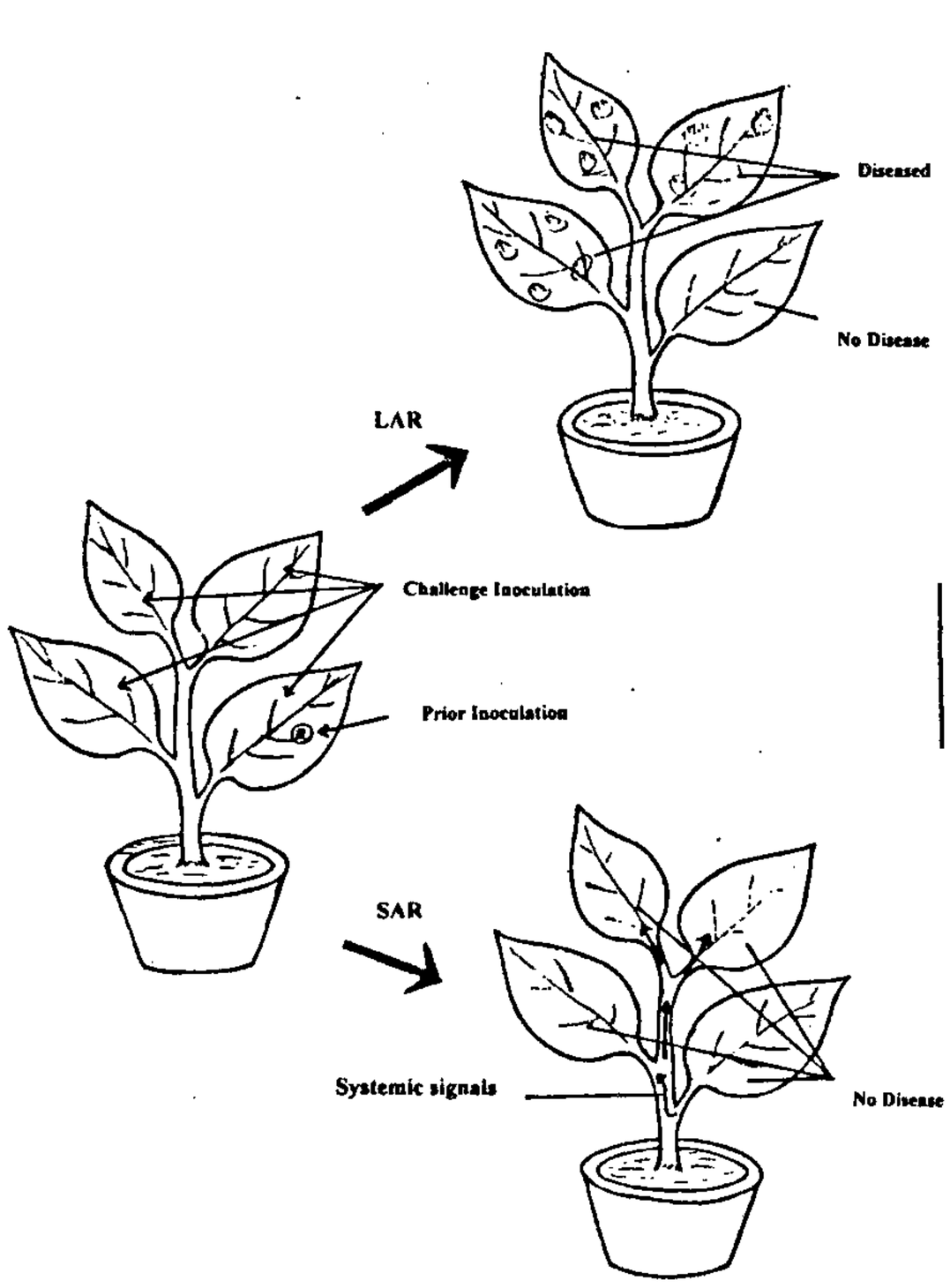

Figure 1. Schematic representation of LAR and SAR in plants. The lower leaf was prior inoculated with biotic/abiotic inducers. Challenge inoculated with the disease-causing organisms. SAR: Systemic Acquired Resistance; LAR: Local Acquired Resistance.

Signaling during systemic resistance

Though for several years signaling in plant diseases has been hypothesized, the concept gained credence only after establishment of SAR. The responses that lead to the activation of SAR can be divided into two phases – *initiation phase* and *maintenance phase*[33]. During the initiation phase, the pathogen infection is recognized and a signal is released to distant tissues for systemic resistance. Apparently, the signal molecule(s) travel through the phloem since studies have shown bi-directional operation of resistance[27]. The systemic signal is perceived by target cells which react by expressing both *SAR* genes and resistance. The maintenance phase refers to the period of time during which the plant is in a quasi steady-state when disease resistance is maintained[34]. During signal transduction, activation of intracellular and intercellular transfer occurs that leads to systemic protection through elicitation of defense compounds[16]. The resistance-inducing agent itself may act as the signal or it may trigger the synthesis of yet unknown signal compounds from the cells of initial necrosis that are translocated systemically in the plant-triggering resistance in adjacent and distant tissues[27]. A compartmentalized compound released due to injury or metabolic perturbation is suggested to serve as the initial signal resulting in the cascade of signals and metabolic

events leading to induced resistance[11]. Such compartmentalized signals function as an alarm signal conditioning an umbrella response to protect the plant against infectious diseases as well as other stresses. In recent years, many types of chemical and nonchemical putative signal molecules have been hypothesized to be involved in the systemic defense response[12,34–38].

Role of plant-derived substances in signal transduction

Application of biochemical, molecular and genetic techniques has identified key components of the signaling pathways leading to defense responses. SA, a benzoic acid derivative has been suggested to be an endogenous signal for SAR as its exogenous application has been shown to induce resistance to a variety of bacterial, fungal and viral pathogens[39,40]. SA is shown to be an endogenous phloem mobile compound that increases in concentration at the onset of SAR in cucumber[41] and *Arabidopsis*[29,42]. Endogenous SA level has been correlated to the induction of PR proteins[40,43]. Also, exogenous application of SA induces the same *SAR* genes that are expressed following biological SAR induction[28,29]. Participation of SA in plant defense has been demonstrated through transgenic plants as well. Studies with transgenic tobacco[44] and *Arabidopsis*[35,45] expressing the *nahG* gene coding for the enzyme salicylate hydroxylase (which converts SA to catechol) from *Pseudomonas putida* demonstrate that the plants that accumulate little SA showed reduced or no *PR* gene expression and fail to establish SAR. In potato expressing the *nahG* gene, induced resistance is strongly decreased, indicating that SA might be a necessary component in the induction of SAR in potato[37]. SA has been observed as a long distance signal in tobacco[46] and cucumber[47] infected with tobacco mosaic virus (TMV) and tobacco necrosis virus respectively. Local and systemic increase in endogenous level of SA in tobacco plants inoculated locally with TMV were observed[48]. Suggestions that SA is not the translocated SAR signal come from the studies on cucumber[49], and also on tobacco through grafting experiments between nahG and wild type[35] and with transgenic tobacco expressing the cholera toxin gene[50]. Also, in a few cases, *PR* gene expression and SAR are recorded in a SA-independent manner as in *Pseudomonas*-induced resistance of *Arabidopsis*[51]. However in a recent report it has been shown that SA along with other compounds accumulate in infected host tissue in response to a signal and elicit defense compounds along with a multitude of compounds[52]. In cucumber inoculated with *Pseudomonas syringae*, the first measurable effect of the mobile signal for SAR is the stimulation of PAL which precedes a transient increase in SA and 4-hydroxybenzoic acid[52]. If SA cannot accumulate, it is shown to certainly block the SAR signal transduction

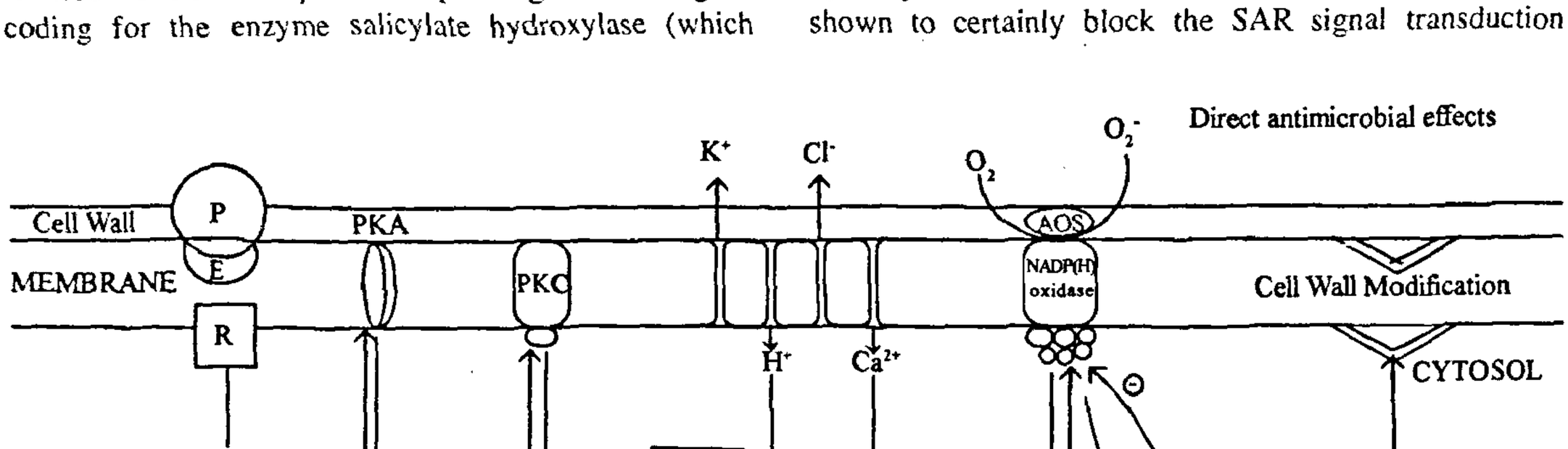

Figure 2. Schematic representation of signal transduction pathway that leads to the expression of defense-related genes in plants. P-pathogen; E-elicitor; R-receptor; PKA-protein kinase A; PKC-protein kinase C; PLC-phospho lipase C; PIP_2-phosphotidyl inositol diphosphate; DAG-diacyl glycerol; cAMP-cyclic AMP.

pathway[35,46,53]. Details on the understanding of the way in which SA is related to SAR come from the studies on the biosynthesis of SA in potato[54] and intermediates of SA biosynthesis in tobacco[55]. In potato, it is shown that synthesis of SA induced by arachidonic acid involves phenylalanine ammonia lyase[54]. Involvement of benzaldehyde and benzyl alcohol promoting SA accumulation and expression of disease resistance is reported in tobacco and they provide insight into the early steps of SA biosynthesis[55].

Plant growth substances like ethylene, jasmonic acid, abscissic acid and systemin have also been identified as defense signals in plants[56–58]. Ethylene, a volatile plant hormone derived from methionine is involved in numerous physiological processes[59]. Production of ethylene upon wounding, pathogen infection and elicitor treatment has been reported[60,61]. Ethylene has been suggested to act as a signal molecule of SAR[60,62], as well. Support for a role for ethylene in disease resistance is based on the following observations – exogenous application of ethylene to tobacco carrying the *N* gene for resistance to TMV results in resistance to TMV as evidenced by the decreased size of necrosis[63], accumulation of PR proteins[39], hydroxyproline-rich cell wall proteins[52], defense-related enzymes such as phenylalanine ammonia lyase, chalcone synthase and vacuolar hydrolases[64] and lignification[52]. However, ethylene application not demonstrating heightened resistance suggests that it may not be directly involved as a signal for disease resistance. In an attempt to clarify the role of ethylene in SAR, it is observed that chemically induced SAR is not an ethylene-dependent process in *Arabidopsis* and ethylene may play a role in SAR by enhancing tissue sensitivity to the action of SA[65]. *SAR* gene expression in ethylene-insensitive mutants of *Arabidopsis* is similar to that in wild type plants[66]. Ethylene has been reported to be the intermediate in SA-induced synthesis of chitinase in tobacco[62] and wound-induced proteinase inhibitor genes in tomato[67], thus supporting the fact that ethylene modulates the expression of resistance. It has been postulated that rhizobacteria-mediated induced systemic resistance in *Arabidopsis* follows a novel signaling pathway in which components from the jasmonate and ethylene response are engaged successively to trigger a defense reaction that is regulated by NPR1 (non expressor of *PR* genes). NPR 1 differentially regulates defense gene expression depending on the signaling pathway that is activated upstream of it[68].

Jasmonates (JA) are derivatives of linolenic acid obtained through the action of lipoxygenase (LOX) mediated oxygenation process. Its methyl ester, methyl jasmonate (Me JA) is volatile and analogous to ethylene in action (for review, see 36). Both JA and Me JA apparently act as second messengers in SAR[69]. Induction of resistance by JA and Me JA has been shown in potato against *Phytophthora infestans*[70], barley against *Erysiphe graminis* f.sp. *hordei*,[71,72] and *Arabidopsis* against *Pythium mastophorum*[38]. Also, treatment of plants with JA and Me JA results in accumulation of antifungal defencin[73], thionine[74], defense-related enzymes[71] and transcripts of jasmonate-responsive defense genes[38]. Similarly, induction of resistance in rice and *Arabidopsis* with their respective avirulent pathogens leads to an increase of endogenous JA[75,76]. It is shown that in monocots, JA and Me JA are not involved in SAR-mediated resistance[71]. Recently, JAs have been suggested to play a role as a master switch for the activation of signal transduction pathway in response to predation and pathogen attack[74,76]. Also, biological relevance of JA-signaling in host–pathogen interaction has been reported[38]. Experiments with *Arabidopsis* mutant has demonstrated that jasmonic acid signaling is essential for protection against the soil-borne pathogenic fungus *Pythium mastophorum*[38].

Abscissic acid (ABA) has been suggested to play a role in the systemic induction of resistance in bean to *Colletotrichum lindemuthianum*[56]. However, local resistance of soybean to *Phytophthora megasperma* and incompatible interaction in soybean and *Phytophthora megasperma* were not associated with increased levels of ABA[77]. Systemin has been reported to be the systemic signal-inducing proteinase inhibitor synthesis after wounding[78,79].

Nonchemical signals in SAR

Long distance electrical signals known as action potentials[80] have been proposed in systemic signaling[81]. According to the hypothesis, ion redistribution across the plasma membrane causes biochemical changes on both sides of the cell, resulting in an action potential during which polysaccharide-degrading enzymes such as polygalacturonic acid lyase, polygalacturonase, pectic lyase and pectin methyl esterase are activated releasing pectic fragments of biological activity. Though this hypothesis is derived from physiological responses of plants in response to wounding (see below), studies with SAR in cucumber support a role for the action potential[82]. In cucurbits, the path for SAR signal movement appears to be transmitted via sieve elements[83]. In tomato plants, transmission of an action potential from the cotyledons to the first leaf in response to wounding has been reported[84]. Also, a strong evidence for electrical signal as the initial signal for the synthesis of proteinase inhibitors in tissues distant from that receiving an injury has been reported[84]. The systemic wound response of several plant species involves the activation of proteinase inhibitor (*pin*) genes and the accumulation of PIN proteins at the local site of injury and systemically throughout the inoculated aerial regions of the plant. Both local and systemic accumulation of *Pin 2* mRNA, due to

application of an electric current, similar to treatment by wounding or heating has been reported[80,85].

Induction of resistance through signaling molecules

A number of events occur at the site of signal generation and these events lead to the generation of various signal molecules. Triggering of systemic response is accelerated by host cell death caused by either the hypersensitive response (HR) or by disease development. In cucumber, rapid induction of acquired resistance is elicited by HR[12]. A minimum efficiency of the potential to cause HR is needed to induce SAR responses. It is found that the signals for the induction of acquired resistance must be generated very early in the HR and before visible death of the tissue[40]. Early physiological events of HR to bacterial pathogens/inducers and their correlation with signal generation for SAR include the generation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2)[86,87]. Evidences suggest that H_2O_2 and other ROS derived compounds act as inducers of defense genes. The ROS may directly kill the pathogen[88], induce cross linking of cell wall proteins[89] and/or enhance lignin synthesis catalysed by peroxidase, thus creating a physical barrier against invading pathogens[90-92]. Also, they may serve as secondary messengers[85] along with diacyl glycerol, inositol triphosphate, Ca^{2+} by inducing the genes of scavenging ROS PR proteins and pathogen tolerance[93,94]. Involvement of ROS in triggering plant defense by causing cell death in soybean suspension cells has been reported[95]. Action of chemical signals may involve binding to a receptor molecule in the plasma membrane[69] or an alternative site in the cell causing specific changes in host metabolism. In tobacco cells, a soluble protein that binds SA has been reported[96]. Studies with SA report inactivation of catalase by SA and increase in endogenous H_2O_2 causing resistance[97]. It has been suggested that one of the early functions of SA is the modulation of signaling pathways that activate a sustained oxidative burst following pathogen recognition and specific exchange of ions across the membrane. Activation of oxidative burst stands temporarily between the earliest events such as the stimulation of ion fluxes across the plasma membrane, proline-rich protein and hydroxyproline-rich protein production and the later changes in gene expression and transcription factor[98]. Avirulence signal receptor protein interactions probably take place in systems where ROS is a product of exocellular matrix, the coupling is probably indirect with at least ion channels acting upstream of the oxidative burst[99]. Localized ion fluxes can be related to the membrane depolarization that accompanies the initial phase of HR cell death[100] and generation of electrical signals. Early in HR, there is a calcium-dependent turn-over of phosphatidyl inositol which gives rise to DAG and IP-3 which facilitate activation of PKC and mobilization of Ca^{2+} ions, suggesting the possibility of generation of lipid-derived signals such as jasmonic acid[101]. Increase in the non-specific permeability of the host cell in relation to the increased HR leads to electrolyte leakages[102], thus affecting the ionic balance of the surrounding cells and aiding the generation of electric signals.

Signal transduction in plants and animals

The eukaryotes are characterized by a highly conserved phospholipase signaling pathway for regulation of pathogenic microbes[103]. These include activation of G protein (trimeric GTP binding) and tyrosine protein kinase activity for receptor–effector coupling proteins[104], phosphoinositide metabolism to respond to extracellular signal[105], activation of calcium channels by a phospholipase C (PLC)-generated second messenger, inositol 1,4,5-triphosphate and involvement of Ca^{++} ions and protein phosphorylation. Signaling activates the host defense system through resistance genes that are multiple, allelic and unstable with genetic loci and formation of protein products that recognize a variety of molecules[106]. One of the earliest changes upon pathogen attack in plants and animals is the rapid increase in ROS[107]. Thus, reactions of plant cells and the vertebrates' immune system, T and B cells, appear to be biochemically similar[108,109]. Involvement of noninducible immunity (*NIM 1*) gene product in the signal transduction cascade leading to gene-for-gene resistance in *Arabidopsis* has been reported[33]. The NIM 1 protein, which shows homology to the mammalian signal transduction factor Ik-B, suggested interaction of NIM 1 with an NF-kB-related transcription factor to trigger disease resistance[32,33]. Also, the deduced amino acid sequence of *N* and *R* gene from tobacco includes a domain that is related to Toll, a regulator of disease resistance responses in *Drosophila*[110,111]. Recently, similarities in pathogen recognition, STP and some defense responses between plants and animals have been reported[112]. These reports suggest that SAR signaling pathway in plants is representative of an ancient and ubiquitous defense mechanism in higher plants[33].

Conclusions

Induced resistance by defense signaling with compounds that have no direct antimicrobial effect is a recent development that provides a novel, environmentally safe strategy for growing healthier crops without pesticides. It is evolving as a new generation crop protection procedure needing a multidisciplinary assessment through coordinated research and development programmes from basic studies on pathogen/host genetics to field trialing.

The nature of systemic signaling in plants is an extremely interesting and rewarding area of research. An understanding of this mechanism for induction and maintenance of resistance has the potential of bringing out new fundamental knowledge as well as development of both novel plant protection measures and genetically engineered plants with enhanced resistance to disease. Identification of signal molecules is useful for the development of disease management techniques using the molecular signal substances itself (sprays or as seed dressing) or by methods triggering these signals. SA, a close relative of aspirin, is a natural inducer of disease resistance of plants. SA activity initiating local and systemic accumulation of defense-related proteins is responsible for increased disease resistance throughout the plant. Methyl salicylate, a major volatile metabolite of SA, produced by pathogen-inoculated parts of the plants can also function as an airborne signal that activates disease resistance in neighbouring plants and in healthy tissues of the infected plants. Though there are reports of phytotoxic effects by some of the elicitors of plant defense signal like SA, certain companies (like Ciba-Geigy) have introduced synthetic elicitors like CGA 245704 for plant protection and have called it 'plant tonic'. However, for some of the disease protection strategies there are no data on full scale toxicity studies of known elicitors on higher animals. Importantly, synthetic elicitors should be differentiated from natural elicitors in terms of resistance or biodegradability in the purview of environmental pollution and food safety. Hence, plant protection regulations are important before applying any of the elicitors for agriculture. There are reports in the literature that demonstrate that induced resistance can work in the field. As induced resistance is broad spectrum and operates through induction of a cascade of resistance rather than by acting on the pathogen, the resistance induced should be sustainable and durable and the pathogens should not develop insensitivity as they do to traditional fungicides which are single metabolic site-directed.

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